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14. ABSTRACT

HER-2/neu (neu) transgenic mice (neu-N mice) overexpress the neu proto-oncogene in a mammary specific fashion and develop spontaneous neu-expressing mammary neoplasia. The neu-N mouse model is among the most informative pre-clinical models available for the development of vaccine strategies for the treatment and prevention of breast cancer. The goal of this proposal was to test the hypothesis that reproductive hormones can modulate immunity to breast cancer antigens in neu-N mice. We explored this hypothesis by assessing vaccine-induced anti-tumor T cell responses at discreet stages in the mouse estrous cycle, as well as during mammary gland involution. Using these assays, we were unable to demonstrate a clear link between anti-tumor immune modulation and reproductive hormones. However, we were able to make very important discoveries regarding the activation and subsequent attenuation of breast cancer-specific CD8+ cytotoxic T cells in response to vaccination. These data provide important insight into mechanisms of immune tolerance that limit anti-tumor immunity.

15. SUBJECT TERMS
tumor immunology, tumor vaccines

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Introduction

While a role for reproductive hormones in breast cancer risk has been an area of intense study, interest in their effects on breast cancer treatment outcomes is only just emerging. Indeed, hormonal influences may underlie the observation that the phase of the menstrual cycle at the time of breast cancer surgery may influence survival (1, 2). In addition to their effects on tumor cell growth and metastasis, reproductive hormones may also influence our ability to induce breast cancer-specific immunity. Reproductive hormones are known to regulate the function of lymphocytes, and can exacerbate autoimmunity in women (3). Although there has been growing interest in the effects of reproductive hormones on autoimmunity, there are currently no data evaluating their influence on breast cancer vaccine efficacy. Our lab is interested in vaccine-mediated immunity against HER-2/neu (neu) in neu transgenic (neu-N) mice, a pre-clinical model for breast cancer vaccine development. We have observed that neu-specific immunity is poorly induced after vaccination in neu-N mice, similar to what is seen in patients with neu-expressing breast cancer. Intriguingly, a small proportion of vaccinated neu-N mice respond vigorously and can reject their tumors. Among a large cohort of neu-N mice given neu-targeted vaccination, we found that animals that responded vigorously were housed together. After controlling for a number of variables, and considering that mice housed within a cage tend to synchronize their estrous cycles, we suspect that the estrous stage at vaccination may influence the animal's ability to mount a potent antitumor immune response.

Body

In order to test the hypothesis that reproductive hormones can modulate immunity to breast cancer antigens, we performed the following series of experiments:

1) Do hormonal changes during estrous in the mouse affect the ability to induce immunity against a foreign antigen? First, we proposed to characterize the generation of immunity to the influenza nuclear protein (NP) in neu-N mice as a function of the stage of estrous. We used intracellular cytokine staining to detect and quantify NP-specific $CD8^+$ T cells (4, 5). Eight-week old neu-N mice in groups of five each were placed into a cage with bedding material from a male neu-N mouse in order to synchronize their estrous cycles. Vaginal swabs were monitored twice daily and mice were vaccinated at the proestrous, estrous, and diestrous stages. Seven days following vaccination, animals were sacrificed and NP-specific CD8+ T cells enumerated by intracellular cytokine staining for IFN-γ released in response to NP peptide. The data demonstrate no detectable difference in the level of NP-specific immunity at any stage (Figure 1A). Similar data were obtained when we measured the induction of neu-specific CD8+ T cells in neu-N mice given neu-targeted vaccination (Figure 1B), though the magnitude of the response to the self-antigen is less. Because mice pass through the estrous cycle so quickly (4 – 5 days), it is possible that the time required to initiate an immune response (approximately three days) is too great to accurately measure differences in immunity as a function of hormone levels. We are currently exploring the use of subcutaneous estrogen tablets as a means to boost hormone levels for an extended period of time. This may provide a more informative analysis.

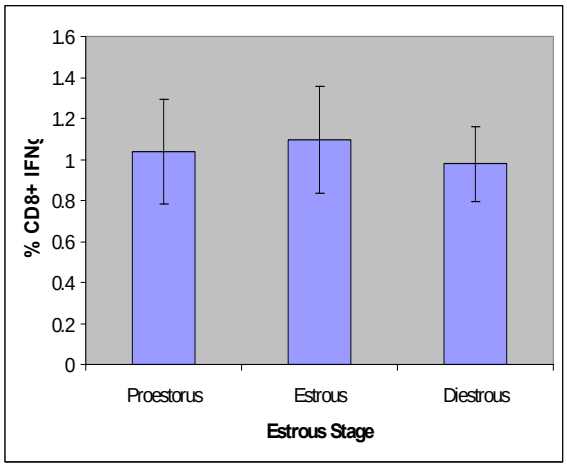

Figure 1A. Immunity to Foreign Antigen.
No evidence of immune modulation as a function of estrous stage is apparent from analyses of CD8+ T cell activatioin against a foreign antigen (NP).

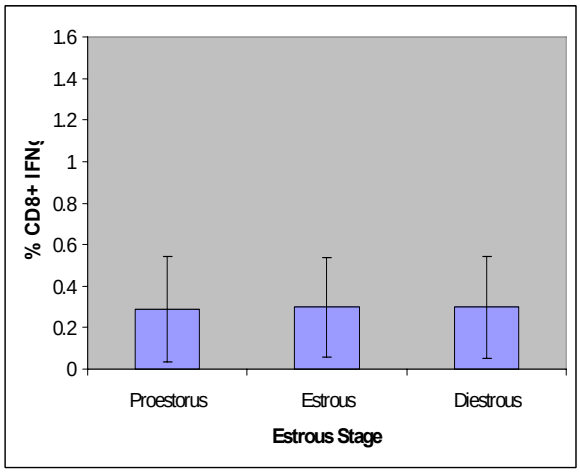

Figure 1B. Immunity to Self-Antigen.
No evidence of immune modulation as a function of estrous stage is apparent from analyses of CD8+ T cell activatioin against a tumor antigen (neu).

2) Is the response to a tumor rejection antigen affected by hormone-associated structural changes within the mammary epithelium? In neu-N mice, neu is expressed in both normal mammary cells and mammary tumors. Apoptosis within the mammary epithelium, induced during mammary gland involution after weaning, may release large amounts of neu protein. Protein released in this way (i.e. in the absence of pro-inflammatory cytokines) would be expected to diminish the response to vaccine. We have evaluated neu-specific T cell responses in the mammary-draining lymph nodes and the spleen (4, 5) after weaning of pups from neu-N breeder females. Again using intracellular cytokine staining, we were able to demonstrate that, although neu-specific immunity overall is slightly lower in post-partum females relative to 8-week-old-virgin females, there was no clear difference between the level of immunity in post-partum females and age-matched virgin females (Figure 2). These data suggest that mammary gland involution has no significant inhibitory effect on vaccine response, but that as neu-N mice age their ability to mount a significant neu-specific immune response may diminish. This likely is the result of repeated exposure to neu protein released during mammary gland involution occurring during the estrous cycle.

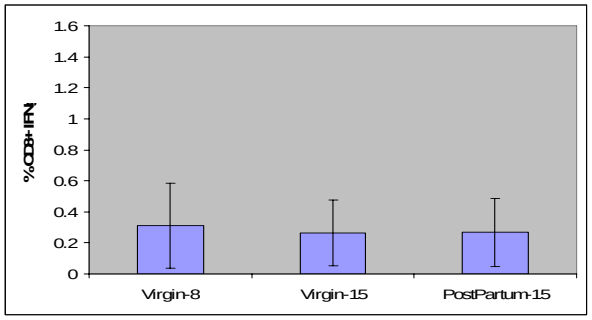

Figure 2. Effects of Age, Involution.
Eight-week-old virgin neu-N mice showed a slightly increased level of neu-specific immunity compared to 15-week-old virgin or post-partum animals. No evidence of post-partum inhibition of neu-specific immunity was seen.

During the course of these studies, we have studies in great detail the timecourse of CD8+ T cell activation in neu-N mice after vaccination with NP-targeted (exogenous antigen) or neu-targeted (self-antigen) vaccination. The data indicate that, while NP-specific immunity develops normally in neu-N mice (not shown), neu-specific CD8+ T cells undergo an abortive activation in response to neu-targeted vaccination (Figure 3A). Furthermore, the data support our earlier observation that neu-specific T cell responses are elicited in only a small proportion of neu-N mice after neu-targeted vaccination (Figure 3B). In preparing this grant proposal, we hypothesized that the activation of neu-specific T cells was occurring only in mice at a specific stage in the estrous cycle (presumable when estrogen levels were highest). The data obtained in the course of the present study do not necessarily dispute our hypothesis, however as a whole the data do not indicate a strong association between estrous cycle and the induction of potent antitumor immunity. Clearly, further study is necessary to definitively explore this issue.

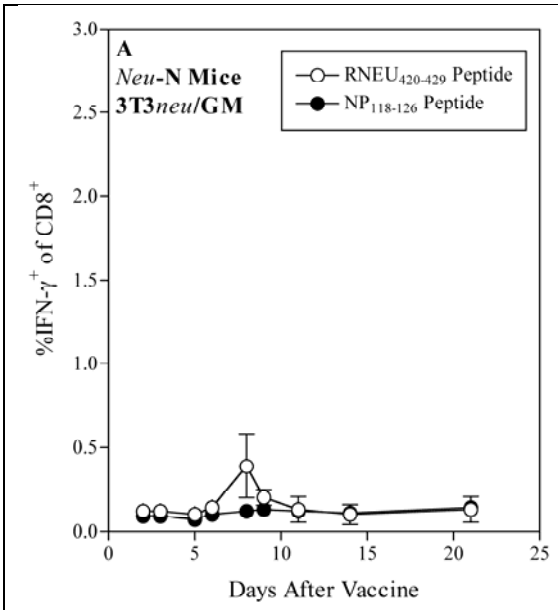

Figure 3A. Timecourse of Neu-specific CD8+ T cell Activation.
Neu-specific CD8+ T cells undergo a brief period of activation, but are undetectable by 14 days after vaccination.

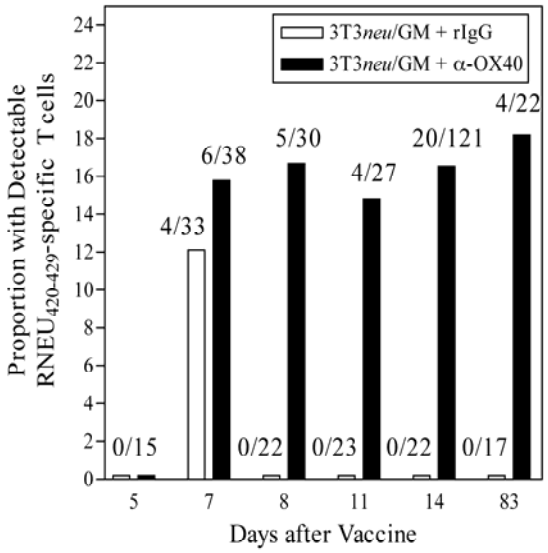

Figure 3B. Frequency of T cell Activation.
Neu-specific T cell activation occurs in only about 15% of vaccinated neu-N mice. With additional costimulation it is possible to promote the persistence activation of these T cells.

Key Research Accomplishments / Reportable Outcomes

We are clearly very disappointed to have generated negative data from this proposal. However, our characterization of the activation of antitumor CD8+ T cells, specifically the fact that these T cells undergo an abortive form of T cell activation, is an important observation. These data imply that it may be possible to alter the course of their activation, pushing them towards cell division and antitumor effector function rather than inactivation. Data obtained using the T cell costimulatory receptor OX40 (see Figure 3B) support this hypothesis.

Conclusions

The data obtained in the course of the present study do not necessarily dispute our hypothesis, however as a whole the data do not indicate a strong association between estrous cycle and the induction of potent antitumor immunity. Clearly, further study is necessary to definitively explore this issue.

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Appendices

None